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The Analysis Of Pathological Crystals By X-ray Diffraction

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THE ANALYSIS OF PATHOLOGICAL CRYSTALS BY X-RAY DIFFRACTION

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Crystalline substances are often found in routine biopsy and autopsy specimens. Normally the occurrence of crystalline material in the body is limited to the inorganic salts of bones ond teeth, but under certain conditions crystals may be observed in soft tissue and in various body-cavities. The latter crystals may be exogenous or endogenous in origin and range from the numerous possible metabolic crystals through the unlimited number of foreign materials which may gain entrance to the body. The indentity of these substances may be of no apparent significance or, as in the case of the pneumoconioses, their analysis may have etiological significance. Usually, the identification of the crystals associated with certain diseases will contribute to the accuracy of the diagnosis, aid in establishing pathogenic conditions, and even at times be a guide in the therapy and prevention of recurrence of these disease conditions.

Chemical methods of analysis can be used in some cases but are often of limited value because of (1) the difficulty of extracting the crystals from the tissue, (2) the minute amount of material available for analysis and (3) the complexity of the chemical processes required. The time required for a skilled chemist to perform such an analysis, when possible, is quite excessive. It is the purpose of this paper to show that x-ray diffraction powder analysis, not being subject to the above limitations, often can provide the desired identification of pathological crystalline materials. The description of the x-ray diffraction powder method has been given in an earlier issue of this *Bulletin*[1] and is more completely described by Klug and Alexander.[2]

METHODS AND MATERIALS

When the pathologist observes the presence of foreign matter in a tissue section, crystallinity of the material usually can be established by observing the birefringence of the particles using polarized light. A thin needle-like sliver of tissue, containing the observed crystals, is cut and inserted into a small thin walled glass capillary tube. Fig. 1, illustrates (A) the tube as received, (B) the shortened capillary after insertion through steel pin and with tissue sliver probed to lower end, and (C) the completed mount after removal of funned-end. It should be noted that tissue which has been subjected to histological stains or mounted in a crystalline medium such as paraffin cannot be used. The obvious reason for this fact is that diffracted lines from the latter extraneous crystalline substances will be added to lines from the unknown crystals in the tissue, thus causing confusion in applying normal methods of pattern interpretation. Usually additional portions of tissue, from which a slide has been prepared, are available. Either fresh or formalin fixed tissue can be used, the latter requiring washing in running tap water for about 15 minutes, in order to remove any of the soluble salts due to the formalin.

The equipment used was a Philips x-ray diffraction source unit (Fig. 2). Each sliver of tissue, mounted as described above was fitted into the central chuck (a)

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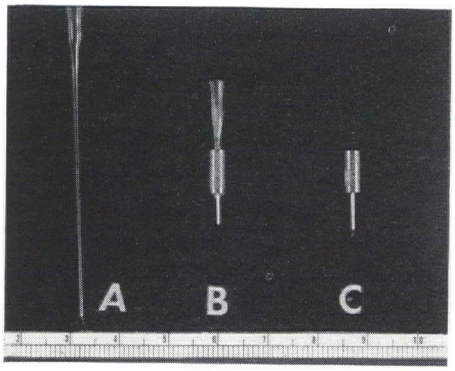

Figure 1

A—glass capillary tube; B—glass capillary tube in pin support, with specimen inserted; C—mounted tube with funnel end removed.

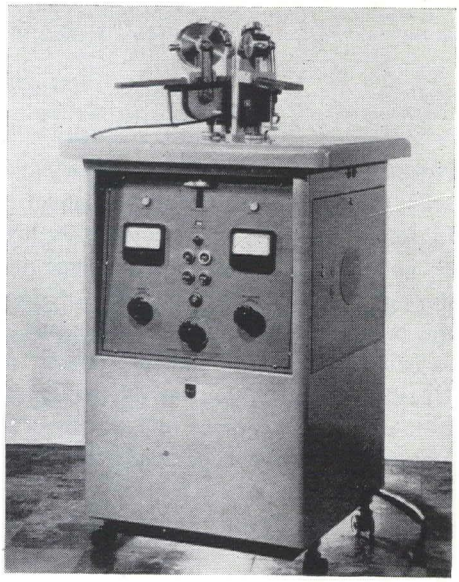

Figure 2

X-ray diffraction source unit with cameras in exposure position.

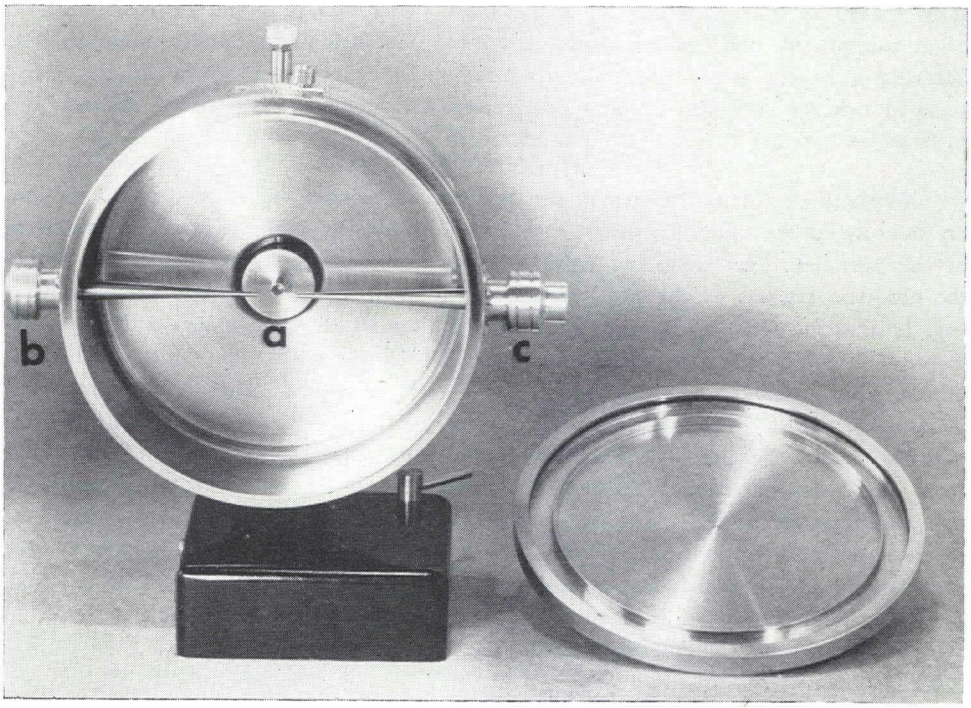

Figure 3

X-ray diffraction powder camera. Specimen is mounted in central chuck (a) in alignment with x-ray beam collimator (b) and beam trap (c).

of the 114.59 mm powder camera (Fig. 3), and adjusted so that it is bathed by the ½ mm beam of monochromatic x-radiation which proceeds from collimator (b) to beam trap (c). Nickel filtered copper K*a* radiation produced at 35 KV and 20 ma was used with the patterns shown in this paper. The exposure time varied from 2 to 4 hours.

REPORT OF CASES

Case 1. The patient was a 58 year old, white man, with a history of angina pectoris, who died following an acute myocardial infarction. At autopsy, the pericardium was thickened, yellow, firm and adherent to the heart by dense fibrous tissue bands (Fig. 4). Microscopic examination of this pericardial mass revealed a foreign body granuloma, containing many giant cells as well as many birefringent or double refractive crystals (Fig. 5). A small sliver of formalin-fixed tissue (washed as described above) was analysed by x-ray diffraction (Plate I, pattern A). This pattern was identical to the pattern obtained from Bakers U.S.P. talc (Plate I, Pattern B). Diagnosis: Talcum powder granuloma, pericardium.

Figure 4

Gross section of fibrotic and adherent pericardium, talcum powder granuloma, Case 1.

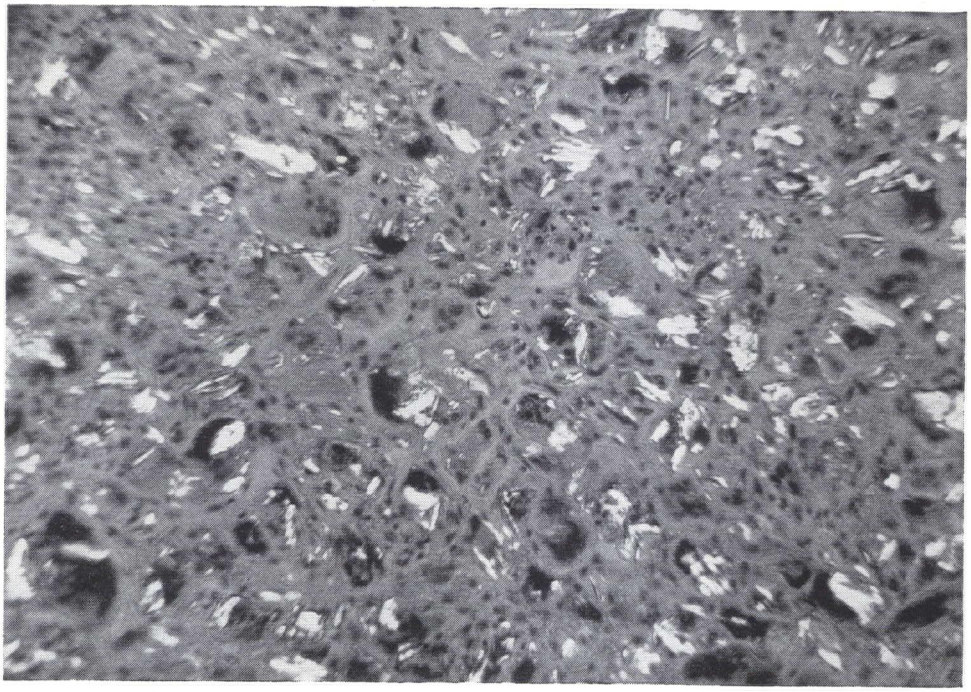

Figure 5
Talc crystals in pericardium, Case 1, partially polarized, H and E stain, X430.

Case 2. This patient was a 43 year old white man with Hodgkin's disease who was treated with x-ray and nitrogen mustard. The patient died 5 years after the initial diagnosis. Autopsy revealed Hodgkin's disease, involving the spleen and lymph nodes. In addition, there was extensive calcification involving the heart, lungs, stomach, kidneys and spleen. The section of the heart, formalin-fixed, was analysed by x-ray diffraction. The resulting pattern (Plate I, Pattern C) was consistent with the pattern obtained from the complex calcium phosphate called apatite. The pattern for mineral apatite is shown in Plate I, Pattern D. When the individual crystallites involved are extremely small, as in the case of the body apatites, the diffraction pattern becomes relatively more diffuse, as can be seen by comparing pattern C with pattern D. Bones and teeth are also composed of apatite as a crystalline lattice.[3] Diagnosis: Calcification, heart (apatite).

Case 3. The patient was a 22 year old white man who developed 2 granulomatous lesions on the right arm (Fig. 6), coincident with the area where he had a tattoo two years prior to admission. The lesions seemed to cover only the red portions of the tattoo and had raised, indurated, purple margins with red granulation tissue centrally. No discharge was present and only moderate tenderness. The ulceration started about six months before admission. The lesion was excised and prepared for microscopic examination. The base of the ulcer was covered by a fibrinous exudate. The underlying granulation tissue extended deep into the subcutaneous fat. Plasma cells, lymphocytes and many eosinophiles were observed microscopically under oil im-

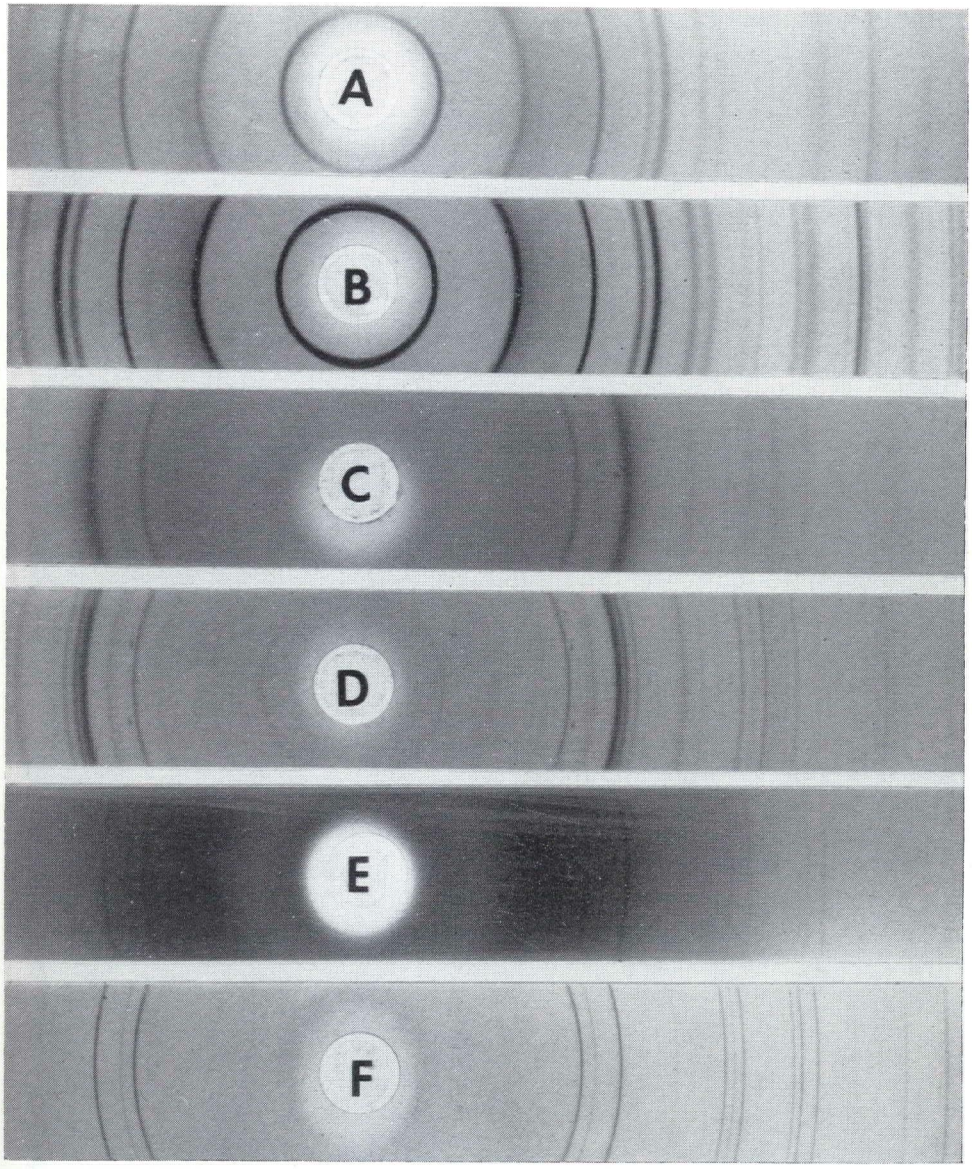

PLATE I

mersion. No giant cells were present. Small collections of reddish brown crystals were scattered throughout the tissue section (Fig. 7). A sample of formalin-fixed tissue from the ulcerated area was analysed by x-ray diffraction (Plate I, pattern E). In spite of the dark band present, due to the scattering by amorphous or non-crystalline tissue, the superimposed lines present in pattern E agree with the x-ray diffraction pattern from C.P. red mercuric sulfide (Merck) (Plate I, pattern F). Diagnosis: Tattoo dermatitis, probably secondary to mercuric sulfide (cinnabar).

Figure 6
Tattoo granuloma, Case 3.

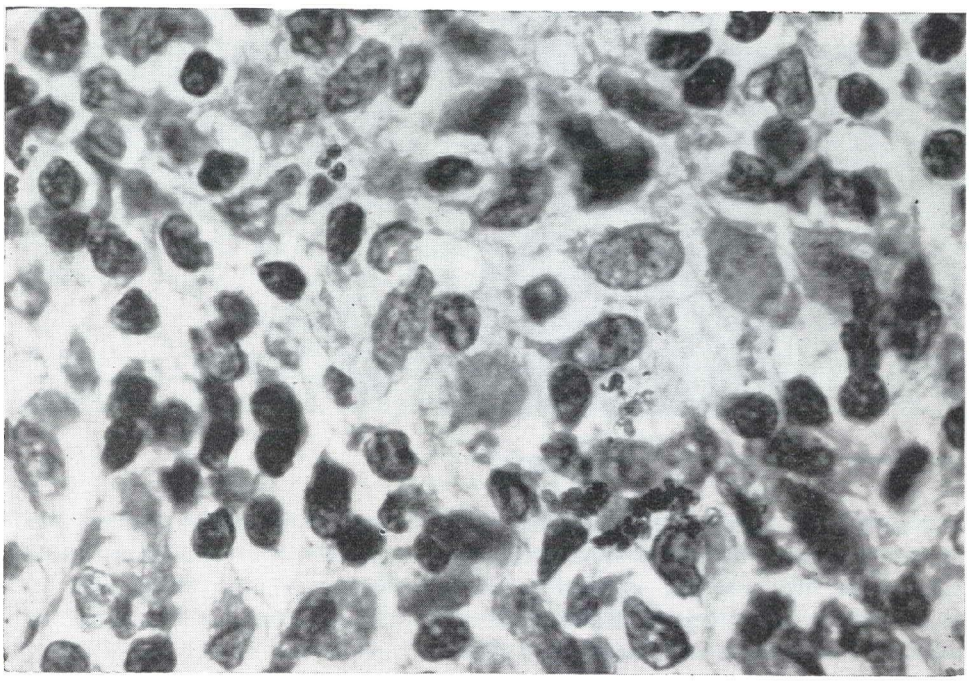

Figure 7
Mercuric sulfide crystals in tattoo granuloma, Case 3, H and E stain, X1100.

Case 4a. The patient was a 53 year old, white man, who died in renal failure, with a clinical diagnosis of collagen disease. He had been a pottery factory worker for 6 years, and had received injections of thorotrast 11 years prior to death as a diagnostic procedure for suspected brain tumor. Microscopic examination of the liver and spleen revealed scattered small deposits of brown crystalline material. In the liver (Fig. 8) these crystals were observed lying free in portal areas, around central veins, and in Kupffer cells. In the spleen the deposits were larger and more numerous than in the liver. Identical x-ray diffraction patterns were obtained from the liver (Plate II, pattern G) and spleen tissue specimens. Comparison of the latter pattern with one obtained from a known thorium dioxide preparation, (Heyden Chemical Corporation, New York) (Plate II, pattern H) shows close basic agreement. Diagnosis: Thorium dioxide deposits (Thorotrast)—liver and spleen.

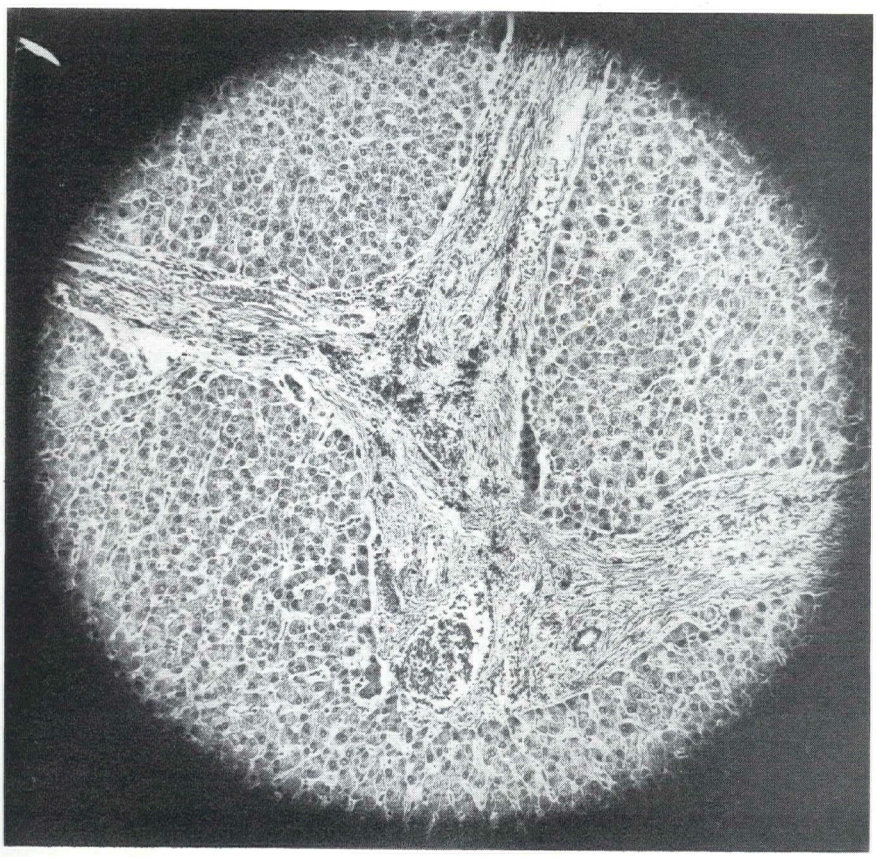

Figure 8

Thorium dioxide deposits in liver, Case 4a, H and E stain, X225.

Case 4b. In the same case (Case 4a), the visceral pleural surfaces and lung parenchyma of all lobes of both lungs were studded with numerous tiny, firm, white nodules, averaging 1 to 5 mm in diameter. Microscopic examination of the nodules revealed dense whorls of acellular hyaline tissue, surrounded by a zone of proliferating

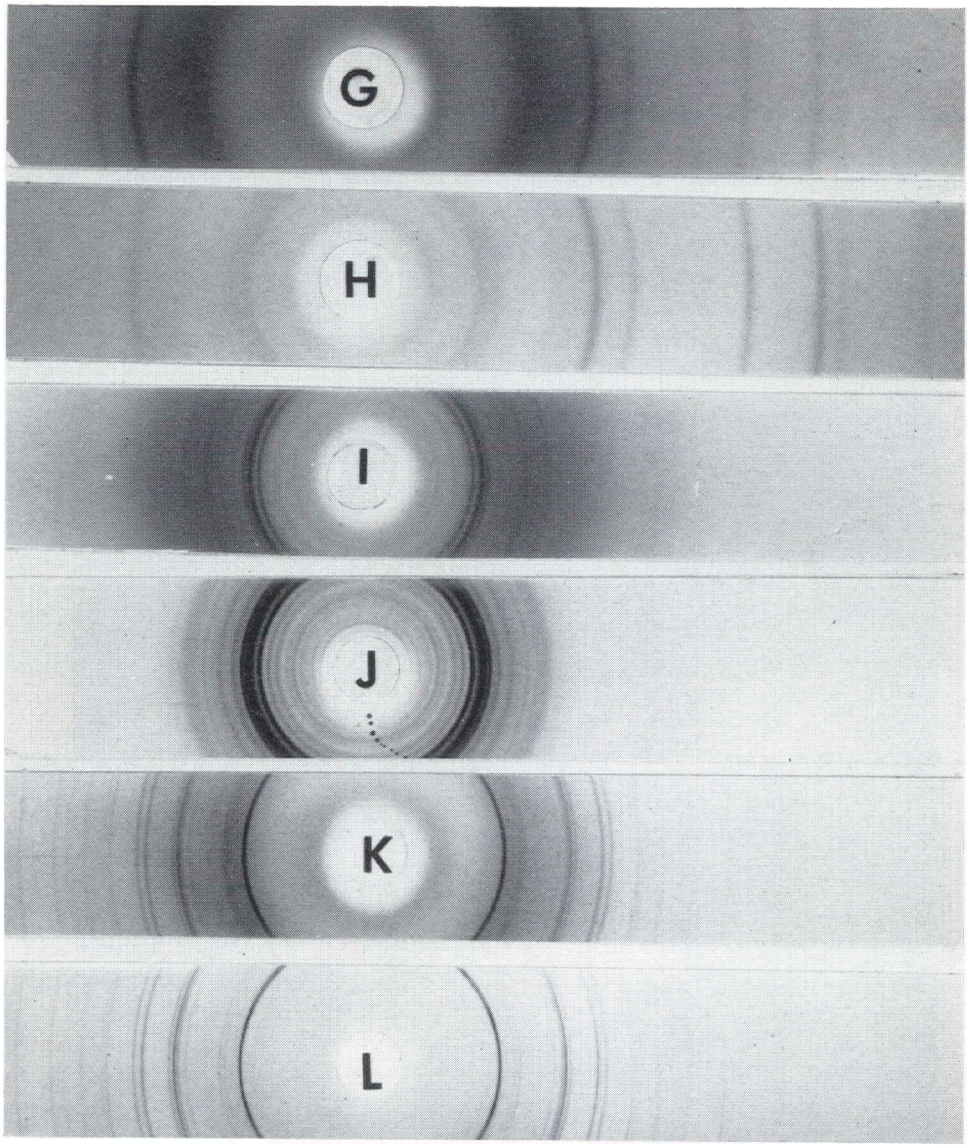

PLATE II

fibroblasts and numerous pigmented macrophages (Fig. 9). Scattered collections of anthrocotic pigment were noted in the central hyaline mass, together with deposits of basophilic material. In the polarizing microscope, crystals were seen both in and around the hyaline mass (Fig. 10). One of the nodules, formalin-fixed, was pressed into the glass capillary glass tube and analysed by x-ray diffraction. The resulting pattern (Plate III, pattern N) was observed to consist of lines characteristic of kaolin (Merck) (Plate III, pattern M) and quartz (A. D. Mackay, Inc.) (Plate III, pattern O). Final diagnosis: Pneumoconiosis, associated with silica (quartz) and kaolin.

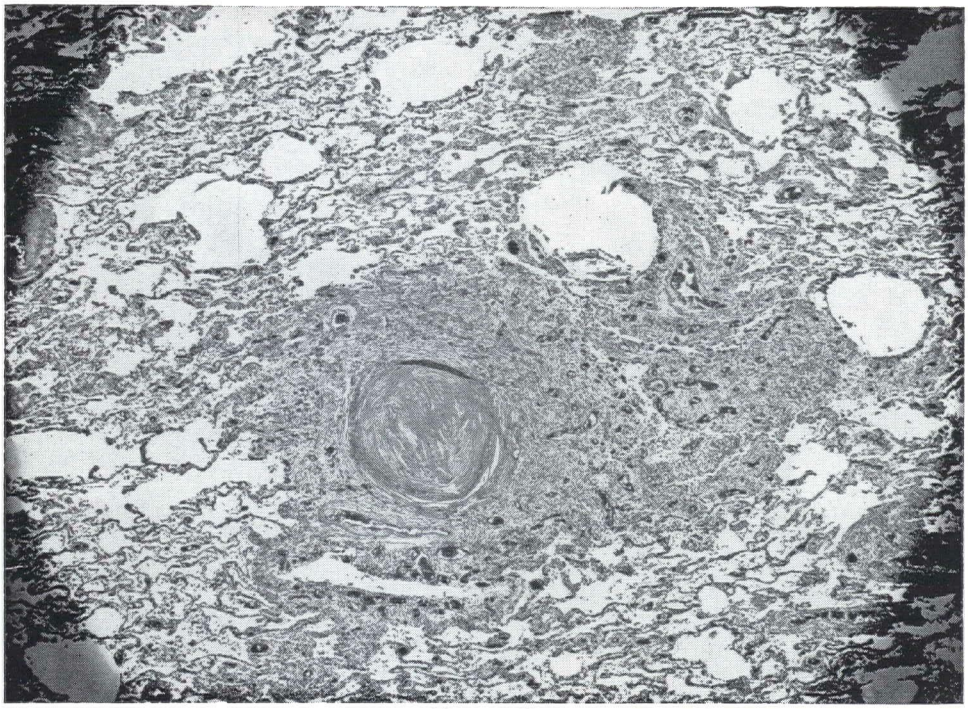

Figure 9

Silicotic lung nodule, Case 4b, H and E stain, X35.

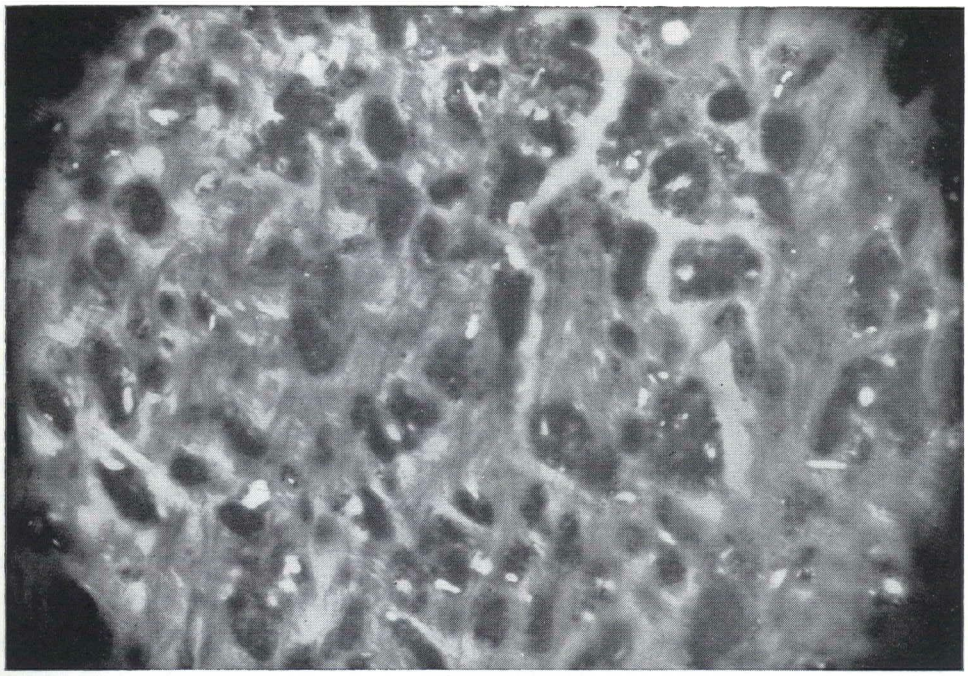

Figure 10

Crystals in lung nodule, Case 4b, in partially polarized light, H and E stain, X730.

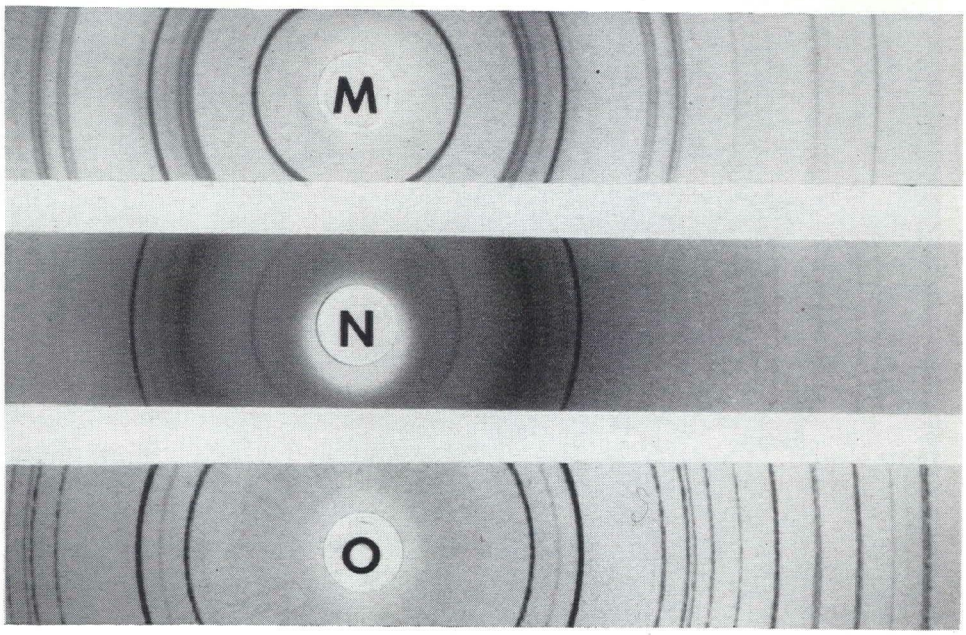

PLATE III

Case 5. The patient, a 4½ year old white female, was brought to the hospital because of severe headaches and vomiting occurring at intervals during the 4 months prior to admission. The general physical examination was normal. The neurological findings included an enlarged cranial vault, marked bilateral papilledema, a mild right facial weakness, and a broad-based gait. Routine laboratory studies were within normal limits. The cerebrospinal fluid protein was 110 mgms. percent. Skull x-rays revealed evidence of increased intra-cranial pressure, and a calcified supracellar mass. Under general anesthesia ventriculography was performed through the coronal sutures. The ventricles were markedly enlarged and under increased pressure. During the exchange of fluid and air, a cystic cavity was encountered and about 90 cc of a oily, xanthrochromic fluid was withdrawn and replaced with air. It was noted that numerous crystals were present in the later cystic fluid, which was sent to the Physics Department for x-ray diffraction analysis. The crystals were removed from the fluid by alternate washing and centrifuging (Fig. 11). Although under the polarizing microscope these crystals gave the appearance of cholesterol, they were definitely established as such by comparison of the x-ray diffraction patterns for the brain crystals and for standard cholesterol (Plate II, Patterns I and J). In this case a ventriculogram revealed an enlarged ventricular system and a huge suprachiasmatic cyst. Through a right frontal craniotomy a large craniopharyngioma was removed. Recovery was satisfactory.

Case 6. The patient was an 18 year old male who has been intermittently hospitalized since birth with the De Toni-Fanconi syndrome, a disorder of the kidney tubules secondary to cystine metabolism. A specimen of lymph nodes was obtained

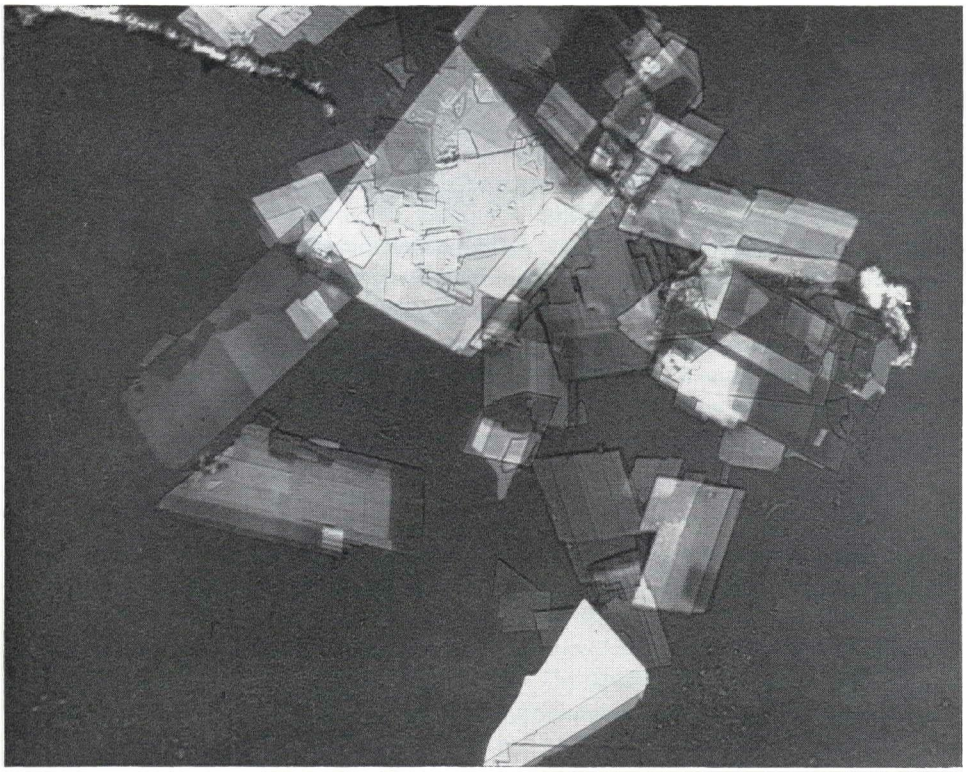

Figure 11

Crystals extracted from intra-cranial cyst fluid, Case 5, taken with partially polarized light, X115.

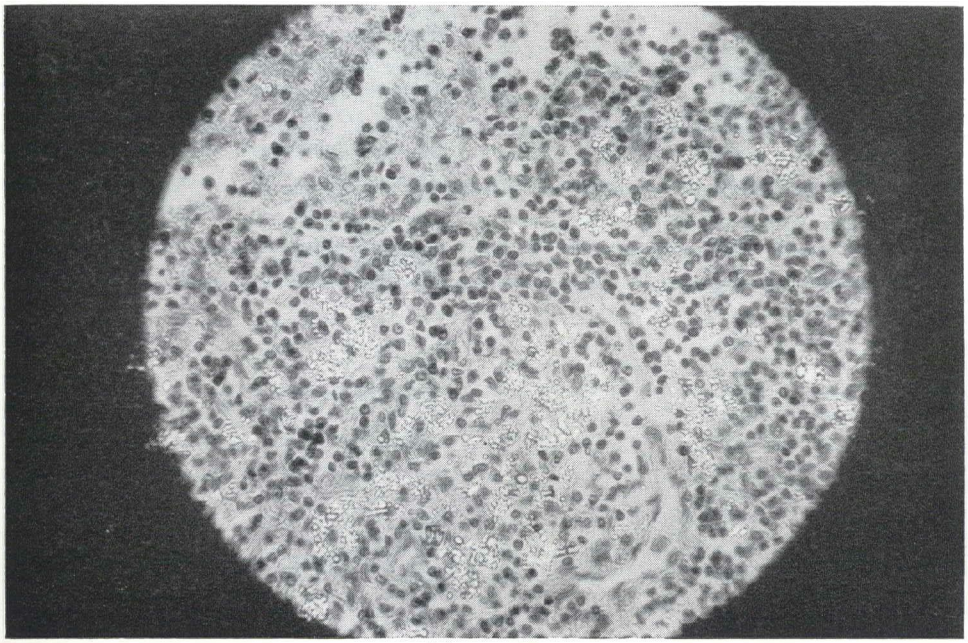

Figure 12

Cystine crystals in lymph node, Case 6, taken with partially polarized light, X600.

from biopsy and sent to the Physics Department for x-ray diffraction analysis when crystals were observed microscopically in partially polarized light (Fig. 12). The diffraction pattern from the lymph node tissue (Plate II,, Pattern K) matched the pattern from standard cystine (Plate II, Pattern L).

DISCUSSION

In illustrating the use of x-ray diffraction in the cases presented, it has been the intent of the author to show only the analysis, by direct visual comparison, of unknown and standard x-ray diffraction patterns. This is the fastest and usually sufficient method of pattern interpretation, when standard patterns of suspected materials are available. When this is not the case, the patterns may be measured and the data

Table I

CASE 4b

Kaolin Standard (Merck) Pattern M, Plate III		Lung Tissue Pattern N, Plate III		Silicon Dioxide (Quartz) Pattern O, Plate III	
d	I/I_1	d	I/I_1	d	I/I_1
7.20	1.00	7.14	0.49		
4.41	0.38	4.42	0.27		
		4.25	0.67	4.26	0.35
4.19	0.38				
3.86	0.15	3.87	0.27		
3.58	0.88	3.58	0.27		
3.39	0.11				
		3.34	1.00	3.34	1.00
3.13	0.08				
2.75	0.05				
2.57	0.28	2.56	0.20		
2.53	0.20				
2.49	0.28	2.45	0.09	2.46	0.12
2.39	0.11				
2.35	0.63	2.34	0.13		
2.30	0.28	2.27	0.13	2.28	0.12
				2.24	0.06
2.20	0.11				
		2.13	0.09	2.13	0.09
2.00	0.20	1.99	0.13	1.98	0.06
1.94	0.11				
1.90	0.15				
1.85	0.15	1.82	0.20	1.82	0.17
1.79	0.15				
1.69	0.11				
1.67	0.20	1.67	0.09	1.67	0.07
1.63	0.15			1.66	0.03
1.59	0.05				
1.54	0.11	1.54	0.13	1.54	0.15
1.49	0.38	1.49	0.09		
1.43	0.08	1.46	0.04	1.45	0.03
1.39	0.03			1.38	0.07
		1.37	0.09	1.37	0.11
1.34	0.11	1.34	0.02		

obtained compared to a published index of x-ray diffraction data.[4] The use of this method is fully described in a previous issue of this *Bulletin*.[1]

When two or more separate crystalline materials are present in an unknown specimen, their individual diffraction patterns appear superimposed upon each other in the unknown pattern. This condition is illustrated in Case 4b where both kaolin and silica (quartz) are present (Plate III, paterns A and C). In such cases it is usually necessary to measure the unknown pattern lines. This has been done for the latter case and the comparative data are presented in Table I. The columns marked "d", in the table, contain the interplanar spacing distances, measured in Angstrom units, for the principal atomic planes present in the crystalline lattice structures involved. The columns marked I/I_1, give the relative intensities of the diffraction lines to other lines in the same crystal pattern. The complete diffraction data for some of these cases, with other cases and data, has been previously published[5] with discussion and literature references to similar cases.

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